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Many methods have been proposed for single cell isolation with or without gadgetry (Hildebrand, *Botan. Rev.*, **16**, 181, 1950). Another procedure is described now, not for any novelty in its principles, but to encourage its wider application in teaching and research.

The micurgical use of an oil chamber has been developed and popularized by de Fonbrune (*Technique de micromanipulation*, Masson Cie, Paris, 1949) primarily as an adjunct to the pneumatic micromanipulator, but the advantages of this chamber for routine purposes need only a trial to be appreciated. For present purposes, the oil chamber merely needs to consist of a clean cover glass or microscope slide, ruled on the reverse in 4 mm squares with india ink. The face of the slide is sterilized in a flame, cooled, and coated with paraffin oil (White Mineral Oil, USP) to a depth of about 0.5 mm. De Fonbrune advises against attempting to sterilize the oil, and my own experience on this advice has been satisfactory. A capillary pipette is drawn by hand from 4 mm glass tubing to a terminal diameter of about 0.1 mm and controlled by mouth by a connecting rubber tube. It is filled partially with the microbial suspension diluted in its growth medium to a density of 10^6 to 10^7 per ml. A drop of 10^{-7} to 10^{-6} ml is deposited then at the center of each square under the oil. This soon spreads to a diameter of 0.1 to 0.2 mm as it adheres to the glass; the flattening provides excellent optical

conditions for the search of each drop by phase contrast, dark field, or low aperture microscopy. The drops that have been verified to contain precisely one cell then are recorded. Immediate recovery of the individual microbes often can be accomplished by repeated flushing of the drops in and out of the capillary pipette. More consistent recovery of clones derived from single cells, approaching 100 per cent with enteric bacteria, is achieved by adding about 10^{-5} ml additional fluid medium to each drop and incubating the slides in a container of oil, e.g., a staining dish, until a large clone develops that can be transferred wherever required by a capillary pipette. The slide can be held safely in a vertical position if the drops are not too large. Uninoculated drops and isolations from known mixtures should be followed, of course, as controls.

A more elaborate chamber for prolonged incubation consists of a rectangular well, built on a slide and filled with oil, over which a cover glass preparation may be inverted. Capillary pipettes drawn from quartz tubing also have proven useful, for they can be sterilized quickly by flaming after a brief rinse to remove surplus solids. The oil "chamber" may be recommended also for any studies that require protracted examination of small culture volumes: cell lineages, motility, agglutination, chemotaxis, and the like. More complicated single cell analyses are done better with micromanipulatory aids, but the present method may find routine applications in any laboratory, whenever doubt arises as to the adequacy of conventional plating methods, to answer questions of clonal purity.

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